We report a 22-year-old male patient with a history of intracranial malignant germ cell tumor (GCT) who had undergone tumor resection twice, followed by radiation and chemotherapy. The tumor had rapidly recurred along the entire ventricular wall with extensive invasion into the brain parenchyma. The serum level of human β-chorionic gonadotropin (β-hCG) was 232.3 ng/ml on admission. Although tissue samples of the recurrent tumor could not be obtained, the previous histological diagnosis of germinoma and elevated serum β-hCG levels suggested recurrence of malignant GCT. The patient declined chemotherapy but accepted dendritic cell (DC)-based immunotherapy. DC inoculation five times resulted in rapid tumor shrinkage and a significant decrease in the serum level of β-hCG. Here we discuss the effectiveness of immunotherapy using DCs for recurrent intracranial malignant GCTs.

Key words: intracranial germ cell tumor – dendritic cell – immunotherapy

INTRODUCTION

Intracranial germ cell tumor (GCT) is a rare neoplasm that affects the central nervous system of children and young adults (1,2). These tumors display a wide range of differentiation and biological malignancy and include mature and immature teratomas, teratoma with malignant transformation, germinoma, yolk sac tumor, choriocarcinoma, embryonal carcinoma and various mixtures of these tumors. Since mature teratoma is curable by surgical resection and pure germinoma is highly radiosensitive, the prognoses of patients with these two categories are favorable. However, the prognoses of patients with other forms are still very poor (1–4). Combination chemotherapy using platinum compounds (cisplatin or carboplatin) improves the treatment results, but most patients with malignant GCTs still suffer multiple recurrences (5).

The dendritic cell (DC) is an extremely potent antigen-presenting cell capable of eliciting strong anti-tumor immunity (6). Recently, immunotherapy using DCs has been attracting attention as a promising therapeutic approach and has been applied for the treatment of patients with B cell lymphoma, malignant melanoma and prostate cancer, showing certain effects (7–9). However, there are still no reports about its effectiveness on brain tumors. The clinical trial of DC-based immunotherapy for brain tumors was approved by the ethical committee of the University of Tokyo and was started in 1997. Here we report a case of recurrent malignant GCT that was treated successfully using DCs.

CASE REPORT

A 22-year-old man with bilateral blindness, paraparesis and difficulty in urination was admitted to the University of Tokyo Hospital on November 20, 1998. He had experienced multiple recurrences of intracranial tumors since initial resection of a pineal teratoma when he was 7 years old and hypothalamic germinoma (Fig. 1) when he was 20. After the partial removal of the tumor, he received radiotherapy (local field, 20 Gy; whole ventricle, 30 Gy) and several series of chemotherapy, which consisted of 20 mg/m² of cisplatin and 60 mg/m² of etoposide daily for five consecutive days. High-dose chemotherapy supported by peripheral blood stem cell transplantation (PBSC) was also administered. These treatments were often effective and although partial remissions could be obtained, cure was not achieved. Therefore, the patient had rejected further treatment for 5 months. During this period, the recurrent tumor had invaded the optic nerve bilaterally, leading to com-
plete blindness and at this time the serum $\beta$-hCG levels, which were within the normal limits until the second operation, started to increase gradually.

At the time of admission, he had large intracranial disseminated masses located along the entire ventricular wall with diffuse invasion into the brain parenchyma. Despite the large tumor, the patient was alert and his neurological function was otherwise normal except for bilateral blindness, paraparesis and urination difficulty. He also had severe diabetes insipidus and general fatigue. At this time, he declined chemotherapy but accepted DC-based immunotherapy and informed consent was obtained from the patient and his parents.

On November 26, DCs were collected by leukapheresis, followed by Ficoll and Percoll density gradient centrifugation (10), with isolation of the DC-rich fraction. After 2 days of culture ($37^\circ\text{C}, 5\% \text{CO}_2$) in medium containing 10% human AB serum, activated DCs were selected by 15% Metrizamide centrifugation, resuspended in saline and infused into the patient intravenously ($2 \times 10^7$ cells). The monocyte-rich fraction (low-density fraction obtained by Percoll centrifugation) was selected and cultured for 7 days in medium containing granulocyte/macrophage colony-stimulating factor (GM-CSF) (1000 IU/ml) and interleukin-4 (IL-4) (500 IU/ml) (11). Thereafter, these monocyte-derived DCs were inoculated into the patient once per week for three consecutive weeks (number of infused cells: $1 \times 10^8, 1 \times 10^8, 2 \times 10^8$, respectively). On December 22, a second session of leukapheresis was performed and DCs ($2 \times 10^7$ cells) were again inoculated as described above (Fig. 2). During the course of the immunotherapy, no other therapies, including steroids, were administered.

As shown in Fig. 3, the intracranial tumor decreased in size progressively and significantly and also the serum $\beta$-hCG levels decreased rapidly (Fig. 4), apparently as a result of this immunotherapy. Unfortunately, however, because of the patient’s difficulty with urination, a urinary catheter was inserted and apparent symptoms of urinary tract infection suddenly appeared on December 28. Despite intensive treatment, he developed septic shock and did not recover. Autopsy was not allowed.

**DISCUSSION**

Intracranial germ cell tumor (GCT) is a rare neoplasm, with a reported incidence in Japan and Western countries of 3.1 and 0.1–3.4%, respectively, among all intracranial tumors (1,2). The prognoses of patients with various histological subtypes except mature teratoma and germinoma are still very poor (3–5). The 2-year survival rate for malignant mixed GCT is only 9% (3). The histology of intracranial GCT, especially germi-
noma, is distinctive, with many T lymphocytes infiltrating among large tumor cells (Fig. 1) (12–14). Examination of T lymphocyte phenotypes revealed the presence of both the cytotoxic/suppressor and helper/inducer T lymphocytes in these tumors, as in other kinds of human neoplasms (14). In spite of this host reaction to the tumor, germinomas usually grow larger if no treatment is given. In contrast, there is a report of a rare case of spontaneous regression of germinoma dependent on the patient’s systemic condition (12). Insufficiency of the antigen presenting system might be one reason for this insecure anti-tumor immunity in germinoma patients. Thus, systemic or local inoculation of potent antigen-presenting cells may be an effective approach for solving this problem.

Among the various immunological approaches, lymphokine-activated killer cell (LAK) therapy has been applied to patients with malignant brain tumors, although its effectiveness is still controversial (15–17). Recent studies (18–20) using a murine model, however, demonstrated that effective anti-tumor immunity could be elicited in the central nervous system by systemic inoculation of tumor antigen-pulsed DCs. In these reports, unpulsed DCs were not effective. On the other hand, it is also known that immature DCs harvested even from cancer patients can be activated by in vitro culture (7,10). Thus, in theory, these systemically inoculated DCs can effectively take up and present tumor antigens and command the tumor-infiltrating lymphocytes in vivo. Supporting this idea, Yang et al. reported that unpulsed DCs could elicit tumor-specific cytotoxic T-cell responses in tumor-bearing mice (21). As far as we know, there are no reports in the literature describing the specific antigen of GCT. Consequently, we could not determine the tumor peptides which bound to the HLA of this patient (HLA-A2/33, B44/51, DR13/14).

In the present case, the previous histological diagnosis of germinoma and the elevated \( \beta \)-hCG level strongly suggested that the patient had germinoma with syncytiotrophoblastic giant cells or elements of choriocarcinoma. After the initiation of immunotherapy, the tumor decreased dramatically and rapidly in size, accompanied by a significant decrease in the level of serum \( \beta \)-hCG (Figs 3 and 4). On examination of peripheral blood lymphocytes, no apparent T-cell clonal expansion was evident, as evaluated by the CD4/CD8 ratio and the T-cell receptor repertoire (data not shown). On the other hand, an increase from 11 to 25% in the peripheral blood NK cell population was observed. The expansion of NK cells may result from stimulation of NK cells by IL-12 secreted from inoculated DCs. Supporting this hypothesis, Fernandez et al. (22) reported recently that DCs were able to stimulate NK cells directly. Hence this NK cell activation and expansion may also be related to the effectiveness of our treatment. As the patient died of disease complications, the long-term outcome of this treatment could not be verified. Nevertheless, the clinical data showed that this new immunological approach is a promising strategy for the treatment of tumors of the central nervous system, especially malignant GCT with a mixed germinoma component.

In this report we described, for the first time, a case of malignant intracranial GCT that was treated successfully by DC-based immunotherapy. Further clinical trials are needed to evaluate the effectiveness and long-term effects of this new approach.
immunotherapy. Since intracranial GCT is a rare neoplasm, these trials should be performed by multi-institutional collaboration. Considering the poor prognosis of patients with intracranial malignant GCTs, it is worth pursuing the possibility of this new immunotherapy in order to increase the treatment modalities available.

References